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GENOTYPE-ENVIRONMENT INTERACTION AND STABILITY OF QUANTITATIVE TRAITS IN GARDEN PEA (*Pisum sativum* L.)

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Abstract

Peas are among the most common and widely cultivated annual legumes. Productivity potential of most modern pea varieties is high but limited by their low homeostasis and sensitivity to abiotic stress, i.e., the varieties tend to reduce adaptability. Therefore, one of the main challenge in pea breeding is to create an optimal genotype capable of realizing the biological potential and adequately responding to changes in growing conditions. Therefore, environmental testing remains relevant. This paper is the first assessment of the breeding samples of the pea working collection (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria) with respect to their ability to form economically significant quantitative traits. Three sources of variability (genotype, environment, and genotype-environment interaction) were found to be statistically significant for the total number of pods, the number of productive nodes with two pods per plant, pod weight, and grain weight. In 2018-2020, the phenotypic stability of ten pea (*Pisum sativum* L.) genotypes was assessed, including four perspective lines (22/16-af, 22/16-n, B4/34-n, and 1/17-n) and six varieties (Kazino-af, Plovdiv-n, Marsy-n, Echo-af, Shugar dwarf-n, and Vecherniza-n). The main examined quantitative traits were the number of pods per plant, the number of fertile nodes with one pod per plant, the number of fertile nodes with two pods per plant, pod length, pod width, pod weight per plant, and grain weight per plant. The effect of all factors of variation on the number of pods per plant, number of fertile nodes with two pods per plant, weight of pods per plant, and grain weight per plant is statistically significant. The strongest was the effect of the environmental factor on the manifestation of the number of pods per plant (52.20 %) and the number of fertile nodes with two pods per plant (59.00 %). The genotype factor has the largest contribution to the total variability of the weight of pods per plant (64.10 %) and grain weight per plant (67.40 %). Therefore, an effective breeding should be focusing on these traits regardless of the environmental conditions. The number of pods per plant and pod length requires more trials to give a more accurate estimate due to the superiority of the genotype×environment interaction variance over the genotype variance. Our findings indicate that the varieties Marsy-n and Echo-af are the most valuable genotypes for the number of pods per plant. The varieties Kazino-af, Plovdiv-n and the line 1/17-ob are highly variable and form fewer pods. For pod weight, all genotypes showed good responsiveness, especially Plovdiv-n (b_i = 2.68), 1/17-ob (b_i = 2.63), and Marsy-n (b_i = 2.18), all three having a higher pod weight, and the Echo-af variety shows better stability (b_i = 1.39; S_i^2 = 1.91). For the grain weight per plant, the Marsy-n (b_i = 3.08), 1/17-ob (b_i = 2.62), and Plovdiv-n (b_i = 4.02) are highly productive but also the most variable.

Keywords: phenotypic stability, genotype, environment, yield stability, ecological plasticity

Peas are among the most widespread and cultivated annual legumes. At the same time, legumes are almost the only source of vegetable protein, which represents about 23-25% of the dry weight of pea seeds [1].

The problem of evaluating the adaptive properties of breeding material is usually solved by experiments that dissect the interaction between the genotype and the environment. As the degree of phenotypic manifestation of the genotype depends on the environment of development, conducting experimental ecological tests, both in time and space, is a real necessity. Data on the productivity of the samples are a reflection of the influence of agro-climatic conditions. These conditions can be much more contrasting and their effect on productivity is much greater than the behavior of the corresponding trait in the conditions of classical testing of varieties [2].

The varietal potential of agricultural crops is one of the main factors for the effective functioning of crop production. Most modern varieties of peas have a high productive potential, the realization of which is limited due to their low homeostasis and sensitivity to abiotic stress [3]. Modern pulse varieties of peas under biotic and abiotic stress (severe drought, excessive moisture, damage from enemies) form 55-72% lower seed weight compared to favorable conditions. In the breeding process there is a tendency to decreasing the adaptive properties of plants to environmental factors, which could become the main reason for reducing the cultivation of this crop [4-6]. In this regard, one of the main tasks facing breeders of this crop is to create an optimal genotype capable of realizing its biological potential and at the same time adequately responding to changes in growing conditions [7)]. The terms “plasticity” and “stability” are used to characterize the potential for modification and genotypic variability of individual traits and plant species. Plasticity, which reflects the variability of traits under different environmental conditions, as well as stability, are considered to be the main adaptive properties of living organisms [8]. The ecological plasticity of the genotype is the ability to stably reach the highest values of the heritable traits under examination in a wide area with sufficiently diverse meteorological conditions [9]. When evaluating varieties of cultivated plants for plasticity and stability of the trait some authors believe that genotypes with medium plasticity and high average trait under different environmental conditions are the best]10]. Others believe that the most promising are the most adaptive genotypes, which are least dependent on the environment and have a high stability of the trait. The third view is that the optimal variety should have a high overall adaptive potential, ensuring maximum yield in both favorable and unfavorable environments [11, 12].

This report presents for the first time the results of studying the ability to form economically significant quantitative traits in breeding accessions from the pea working collection (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria). Three statistically significant sources of variability, the genotype, environment, and genotype-environment interaction were identified for the total number of pods, the number of productive nodes with two pods per plant, the mass of pods, and the mass of grains per plant.

The aim of the study was to evaluate the phenotypic stability of quantitative traits related to productivity in pea genotypes.

Materials and methods. The study was conducted with a garden pea during two consecutive years 2018-2020 at Maritsa Vegetable Crop Research Institute - Plovdiv, Bulgaria. Ten garden pea genotypes from the collection of Maritsa Vegetable Crop Research Institute (Plovdiv, Bulgaria) were chosen as objectives of the present study. Three of them (line 2-22/16-af, 3-Kazino-af. and 5-Echo-af) had afila leaf type, while the other seven (line 1-22/16-n, 4-Plovdiv-n, 6-Marsy-n, 7-Shugar dwarf-n, 8-line B4/34-n, 9-line 1/17-n and 10-Vecherniza-n) possessed normal leaf type. Line 1-22/16-n and line 2-22/16-af were F_{10} generation of the cross Plovdiv × Kazino. 4-Plovdiv-n, 6-Marsy-n, 9-line 1/17-n and

10-Vecherniza-n were varieties developed at the Maritsa Vegetable Crop Research Institute, while 3-Kazino-a., 5-Echo-af, 7-Shugar dwarf-n and 8-line B4/34-n were received through non-cash exchange from the Institute of Plant Genetic Reassures in Sadovo, Bulgaria.

Seeds of the ten genotypes were sown in the field in the second half of March on a high flatbed by scheme 80 + 20 + 40 + 20/4-5 cm (high 4-row bed, 160 cm width). The seeds were planted in two couples of double rows 40 cm apart. The distance between the seeds in the row was 4-5 cm, and the distance between the rows in the couple was 20 cm. The experiments were laid out in a randomized complete block design with three replicates. Plot size was 1.6×4.0 m with 20 seeds in a metre in a row.

The following quantitative features were considered: number of pods per plant (NPP); number of fertile nodes with one pod per plant (NFN-1); number of fertile nodes with 2 pods per plant (NFN-2); pod length (PL), cm; pod width (PW), cm; total weight of pods per plant (WPP), g; grain weight per plant (WGP), g.

The data obtained were processed by two-way analysis of variance ((two-way ANOVA) for each trait to determine the effects of genotypes (G), environment (E) and the genotype-environment interaction (G×E). The assessment of the ecological stability was performed by applying regression analysis according to S.A. Eberhart and W.A. Russel [10] and G.C.C. Tai [13], in which the regression coefficient (b_i, a_i) and the variance of the regression deviations ($Sd^2{}_i$, λ_i) were calculated. The stability parameter (Di) of W.D. Hanson [14] was calculated, which uses the minimum slope of the regression line by the method of K.W. Finlay and G.N. Wilkinson [12]. Analysis of variance was applied to assess average dispersion component (θi) according to R.I. Plaisted and L.C. Peterson [15]; ecovalence (W_2) was estimated by G. Wricke [16] and P. Annicchiarico [17] methodw. The P. Annicchiarico method offers a reliability index (Wi), which estimates the probability that a genotype (variety) will perform lower than the average for the environment or below any standard used. In the nonparametric analysis, the parameter Pi according to the model of C.S. Lin и M.R. Binn [18] and ranking (R) of the samples by adaptability (A) according to the methods of M. Nascimento et al. [19] and M. Huehn [20, 21]. A GGE biplot model was fitted, which uses singular value decomposition of first two principal components [22]. All experimental data were statistically processed using the computer software GENES 2009.7.0 for Windows XP as described [23]. Means (*M*) and standard deviations (±SD) are shown. Differences between the means were assessed by Student's *t*-test and considered statistically significant at $p < 0.05$

Results. Table 1 submeets the main characteristics of the studied genotypes.

1. Basic information about the pea (*Pisum sativum* L.) samples included in the experiment (*M*±SD, Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020)

Genotype	NPP	NFN-1	NFN-2	PL	PW	WPP	WGP
22/16-ob	11,59±1,30^{ab}	2,45±0,55^{ab}	4,56±0,43^{bc}	7,30±0,19^{de}	1,17±0,21^{bc}	36,66±0,70^{a}	14,23±0,97^{a}
22/16-af	10,00±1,12^{ab}	2,92±0,66^{ab}	3,61±0,51^{ab}	6,69±0,22^{bc}	1,01±0,25^{a}	26,23±0,84^{a}	10,36±0,97^{a}
Kazino-af	11,00±1,23^{ab}	2,79±0,6^{ab} 3	4,24±0,49^{abc}	6,56±0,21^{bc}	1,03±0,24^{ab}	30,89±0,80^{a}	12,72±0,93^{a}
Plovdiv-n	10,93±1,23^{ab}	3,62±0,82^{b}	3,65±0,64^{ab}	6,053±0,27^{a}	1,03±0,31^{ab}	32,20±1,04^{a}	15,32±1,20^{a}
Echo-af	12,00±1,35^{ab}	2,24±0,51^{a}	4,94±0,39^{bc}	6,09±0,17^{ab}	0,93±0,19^{a}	25,10±0,64^{a}	12,33±0,74^{a}
Marsy-n	13,69±1,54^{b}	2,95±0,67^{ab}	5,40±0,52^{c}	7,78±0,22^{e}	1,05±0,25^{ab}	56,48±0,85^{b}	26,27±0,98^{b}
Shugar dwarf-n	12,00±1,35^{ab}	3,62±0,82^{b}	4,42±0,64^{abc}	6,66±0,27^{abc}	1,22±0,31^{c}	31,49±1,04^{a}	13,14±1,20^{a}
B4-34-n	12,00±1,35^{ab}	2,59±0,59^{ab}	4,67±0,46^{bc}	6,88±0,20^{cd}	1,17±0,22^{bc}	30,59±0,74^{a}	10,60±0,86^{a}
1/17-ob	11,00±1,23^{ab}	1,78±0,40^{a}	3,01±0,31^{a}	6,74±0,13^{d}	1,05±0,15^{ab}	30,64±0,51^{a}	14,83±0,59^{a}
Vechernitza-n	9,00±1,01^{a}	2,05±0,46^{a}	3,55±0,36^{ab}	6,61±0,16^{abc}	0,97±0,17^{a}	23,66±0,59^{a}	10,53±0,68^{a}
Mean±SD	11,32±1,27	2,70±0,61	4,20±0,74	6,74±0,51	1,06±0,09	32,39±9,28	14,03±4,66

Note. NPP means the number of pods per plant; NFN-1 means the number of fertile nodes with one pod per plant; NFN-2 means the number of fertile nodes with 2 pods per plant; PL means pod length, cm; PW means pod width, cm; WPP means total weight of pods per plant, g; WGP means grain weight per plant, g.

^{abcd} Different letters mean statistically significant differences at $p < 0.05$.

Agrometeorological conditions for the study period are represented by the average daily air temperature and the amount of precipitation (Table 2). The average amount of precipitation is characterized by a pronounced maximum in April 2020 (76.0 l/m^2) and especially in the third ten days of June 2018, as well as the first ten days of June 2019 (125 and 108 l/m^2, respectively). The average daily temperature in March, April, May and June ranges from 4.6 °C to 24.8 °C. The lowest air temperature was recorded in March 2018 and in April and May 2020. The month of May is characterized by lower temperatures in 2019 and 2020, when stronger deviations are observed, while in 2018 they are relatively constant. The average values of meteorological factors have shown a favorable combination with each other for 2020, which had a positive effect on plant development.

2. Characteristics of meteorological elements in different months during the vegetation period (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria)

Decade/10-day period/month	Average temperature for ten days, °C			Rainfall for ten days, l/m^2		
	2018	2019	2020	2018	2019	2020
I/1-10/03	4.62	11.48	10.02	8.00	1.00	21.00
II/11-20/03	10.71	11.49	9.57	15.00	8.00	21.00
III/21-30/03	6.75	10.53	8.22	42.00	0.00	61.00
I/1-10/04	14.04	10.89	7.88	18.00	45.00	76.00
II/11-20/04	16.05	11.86	13.97	2.00	35.00	16.00
III/21-30/04	18.32	14.67	12.75	1.00	8.00	25.00
I/1-10/05	18.92	15.26	15.54	2.00	2.00	14.00
II/11-20/05	19.64	17.71	21.97	9.00	9.00	0.00
III/21-30/05	19.35	20.99	15.66	21.00	59.00	24.00
I/1-10/06	23.80	20.86	20.02	3.00	108.00	15.00
II/11-20/06	24.10	24.83	21.04	10.00	11.00	34.00
III/21-30/06	21.00	24.03	23.29	125.00	41.00	2.00

3. Mean squares (MS) **from the two-way analysis of the variance of 10 samples of peas (*Pisum sativum* L.) for seven traits** (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020)

Source of variation	df	MS						
		NPP	NFN-1	NFN-2	PL	PW	WPP	WGP
Environment (E)	2	33.3969***	14.3263***	13.3668**	1.9813	0.2212	241.9320***	43.0120***
Genotypt (G)	9	13.3116***	3.3788*	4.8911**	2.379*	0.0786	775.2542***	195.0155***
G×E	18	17.2714***	1.6588	4.39401*	6.8851	0.3845	192.5514***	51.3245***
E/G	20	18.884***	2.9256*	3.5337	0.5424	0.0413	197.4895***	50.4933***
E/G-1	2	6.3693*	2.2789	7.8776*	0.6962	0.0728	167.8477***	58.0156***
E/G-2	2	14.3731***	0.9139	6.365	1.8486	0.0152	73.3264***	5.7927**
E/G-3	2	43.9108***	0.5971	1.73642**	0.0648	0.0386	315.4953***	48.1701***
E/G-4	2	47.0667***	3.5308	1.44234**	2.1878	0.0294	550.1775***	192.2619***
E/G-5	2	3.6688*	5.7229	8.5688*	0.0338	0.0224	50.2644***	22.8852***
E/G-6	2	3.0963	3.4624	3.7374	2.2838	0.1226	118.9497***	56.0016***
E/G-7	2	6.1129*	2.0293	2.0486	2.8334	0.1568	221.9331***	49.0267***
E/G-8	2	10.9084***	3.1261	4.2422	0.2178	0.3042	160.1757***	33.3229***
E/G-9	2	50.1025***	3.7525	5.1368	0.5432	0.0234	237.2773***	33.8647***
E/G-10	2	3.2311	3.8416	0.9098	0.1382	0.0416	79.4469***	5.5911**
Residual	29							

Npte. G-1 — 22/16-ob, G-2 — 22/16-af, G-3 — Kazino-af, G-4 — Plovdiv-n, G-5 — Echo-af, G-6 — Marsy-n, G-7 — Shugar dwarf-n, G-8 — B4-34-n, G-9 — 1/17-ob, G-10 — Vechernitza-n; NPP means the number of pods per plant; NFN-1 means the number of fertile nodes with one pod per plant; NFN-2 means the number of fertile nodes with 2 pods per plant; PL means pod length; PW means pod width; WPP means total weight of pods per plant; WGP means grain weight per plant.

*, **, *** The influence of the factor is statistically significant at $p = 0.1$, $p = 0.05$ and $p = 0.01$, respectively,

ANOVA. The results of the two-factor analysis of variance (Table 3) show that there are significant differences between the pea samples on almost all traits except pod width (PW). The genotypic differences were found insignificant for this triat. The influence of the environment was statistically reliable for the following parameters: total number of pods is reliable; number of fertile nodes with one pod per plant; number of fertile nodes with 2 pods per plant; pod weight and grain weight per plant. According to the analysis of the variance, the factor genotype×environment interaction has a significant influence on the total number of pods, number of fertile nodes with 2 pods per plant, pod weight and grain weight per plant.

The values of the sum of the squares (SS) of the traits analysis were used to determine the contribution of each source of variation in the total variability. The variation of the indicators total number of pods (52.20%) and number of fertile nodes with 2 pods per plant (59.00%) is mostly due to the environment, and the influence of genotype and genotype-environment interaction is significantly less (Fig. 1). The largest contribution of the total variability of the traits pod weight (64.10%), grain weight (67.40%) is due to the genotype factor. Therefore, an effective breeding can be done for these traits, regardless of the environmental conditions. The part of the total variation due to the genotype-environment interaction is greater than that resulting from the influence of the genotype factor on the traits total number of pods and pod length (PL), taking into account the insignificance of the genotype-environment factor for the second indicator. The obtained results show that longer-term studies are needed to establish the ecological stability of these traits. Statistically significant effect of all three factors of variation of such traits as total number of pods; number of fertile nodes with 2 pods per plant; pod weight and grain weight is a prerequisite for determining their stability during the study period.

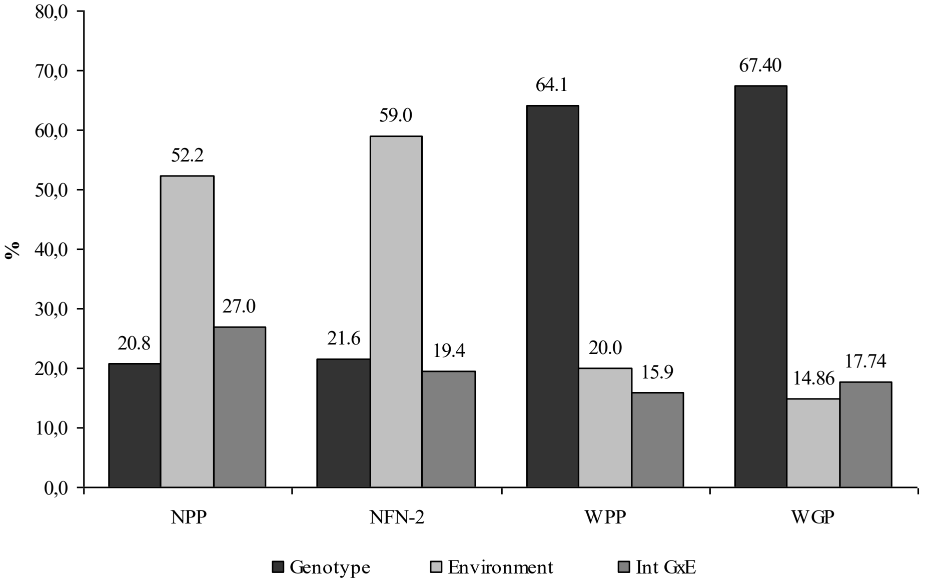

Fig. 1. Percentage impact of the factors genotype, environment and genotype×environment interaction on the general variation of the studied traits in 10 samples of peas (*Pisum sativum* L.). NPP means the number of pods per plant; NFN-2 means the number of fertile nodes with 2 pods per plant; WPP means total weight of pods per plant; WGP means grain weight per plant (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020). For a list of samples, see the Materials and methods section.

Stability parameters. The indicators of S.A. Eberhart и W.A. Russel [10] и G.C.C. Tai [13] for ecological plasticity and stability allow assessing the adaptability of the samples to the specific conditions of the growing environment. The plasticity of the genotypes is calculated by the coefficients bi and ai, and their stability according to the variance of the stability of the trait (S_i^2 and λ_i).

4. Phenotypic stability of the main productivity traits in the studied pea (*Pisum sativum* L.) samples based on regression analysus (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020)

Sample	S.A. Eberhart и W.A. Russel [10]		G.C.C. Tai [13]		S.A. Eberhart и W.A. Russel [10]		G.C.C. Tai [13]		S.A. Eberhart и W.A. Russel [10]		G.C.C. Tai [13]		S.A. Eberhart и W.A. Russel [10]		G.C.C. Tai [13]	
	b_i	S_i^2	a_i	λ_i	b_i	S_i^2	a_i	λ_i	b_i	S_i^2	a_i	λ_i	b_i	S_i^2	a_i	λ_i
	NPP				NFN-2				WPP				WGP			
22/16-ob	1.25	0.44	1.26	1.28	1.64	–0.10	1.69	0.33	0.61	105.52**	0.61	176.41	0.46	37.72***	0.45	63.40
22/16-af	2 .068	0.27	2.10	–0.03	1.47	–0.13	1.50	0.32	1.61*	6.51**	1 .61	11.38	1.03	0.49	1.03	1.37
Kazino-af	1.13	26.10**	1.13	44.06	0.74	4.96**	0.72	8.82	1.82**	156.14**	1.83	260.73	1.40	26.16***	1.41	44.14
Plovdiv-n	2.96***	11.44**	3.03	19.19	2.05	0.71	2.14	1.61	2.68**	250.25**	2.69	417.33	4.02***	81.33***	4.10	135.07
Echo-af	0.52	1.52*	0.50	3.06	1.32	0.96	1.35	2.14	1.39	1.91*	1.39	3.73	1.23	10.58***	1.24	18.19
Marsy-n	0.17	1.66*	0.15	3.25	0.96	0.09	0.96	0.71	2.18**	1.76*	2. 19	3.34	3.08***	9.69***	3.14	16.21
Shugar dwarf-n	–1.02	1.43*	–1.08	2.48	–0.84	–0.28	–0.99	–0.32	–1.29**	120.61**	–1.30	200.99	–2.49***	14.47***	–2.58	23.28
B4-34-n	0.40	6.58**	0.38	11.49	0.88	0.39	0.87	1.21	–2.44**	9.98**	–2.46	15.87	–2.45***	4.62**	–2.54	6.91
1/17-ob	2.51	19.05**	2.55	32.06	1.35	–0.25	1.38	0.12	2.63**	46.06**	2.64	77.04	2.62**	2.41**	2.67	4.28
Vechernitza-n	0.01	1.82*	–0.02	3.48	0.42	–0.19	0.38	0.20	0.79	42.58**	0.79	71.52	1.08	0.02	1.09	0.59

Note. NPP means the number of pods per plant; NFN-1 means the number of fertile nodes with one pod per plant; NFN-2 means the number of fertile nodes with 2 pods per plant; PL means pod length; PW means pod width; WPP means total weight of pods per plant; WGP means grain weight per plant.

*, ** Coefficients are statistically significant at $p = 0.05$ and $p = 0.01$, respectively.

5. **Phenotypic stability of the main productivity traits in the studied pea (*Pisum sativum* L.) samples based on dispersion analysus** (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020)

Образец	R.I. Plaisted и L.C. Peterson [15]	G. Wricke [16]	P. Annicchiarico [17]	R.I. Plaisted и L.C. Peterson [15]	G. Wricke [16]	P. Annicchiarico [17]	R.I. Plaisted и L.C. Peterson [15]	G. Wricke [16]	P. Annicchiarico [17]	R.I. Plaisted и L.C. Peterson [15]	G. Wricke [16)]	P. Annicchiarico [17]
	PP	W^2	W_i	PP	W^2	W_i	PP	W^2	W_i	PP	W^2	W_i
	NPP			NFN-2			WPP			WGP		
22/16-ob	2.80	2.73	97.85	0.24	1.78	99.45	61.84	324.86	98.72	19.02	116.66	81.39
22/16-af	3.27	7.80	80.63	0.18	1.20	75.83	35.35	38.81	74.40	8.45	2.47	70.22
Kazino-af	9.90	79.42	78.41	1.56	16.07	77.17	78.29	502.56	76.65	15.71	80.86	73.38
Plovdiv-n	8.21	61.18	76.09	0.64	6.11	67.37	114.07	888.99	73.53	38.21	323.84	73.52
Echo-af	3.21	7.13	100.43	0.46	4.16	105.03	33.07	14.18	72.81	11.30	33.21	76.72
Marsy-n	3.52	10.58	111.79	0.19	1.27	120.89	38.66	74.56	170.71	14.47	67.51	173.84
Shugar dwarf-n	5.56	32.51	93.14	0.93	9.22	85.76	88.93	617.48	77.17	22.07	149.62	71.21
B4-34-n	4.69	23.20	94.28	0.28	2.22	102.07	87.81	605.40	74.72	19.10	117.45	56.30
1/17-ob	9.34	73.38	70.46	0.13	0.58	62.26	56.59	268.17	79.66	11.10	31.09	93.85
Vechernitza-n	3.75	12.96	74.94	0.20	1.31	78.71	43.88	130.90	63.76	8.33	1.13	72.07

Note. NPP means the number of pods per plant; NFN-2 means the number of fertile nodes with 2 pods per plant; WPP means total weight of pods per plant; WGP means grain weight per plant.

According to the data in Table 1, the variety Marsy-n forms the largest number of pods per plant, approx. 13-14. According to the methods of S.A. Eberhart и W.A. Russel [10] и G.C.C. Tai (13), it is difficult to interpret the plasticity of the number of pods, given the significance of the regression coefficient only for the variety Plovdiv (b_i = 2.96) (Table 4). The low values of the S_i^2 parameter for Shugar dwarf, Echo-af and Marsy-n characterized them as ecologically stable. Three varieties (Vechernitza (S_i^2 = 1.82). Kazino-af, 1/17-ob and Plovdiv), which form a number of pods below the average for the test group, can be referred to as the group of highly variable and unstable genotypes. Most of the variance-based parameters such as PP [15] W^2 [16] define 22/16-ob as the genotype with the highest ecological stability of the number of pods, followed by Echo-af (Table 5).

The situation is similar for the values of the parameters based on the regression analysis for the number of fertile nodes with 2 pods per plant (see Table 4). Therefore, other methodological approaches may be applied to characterize the stability of this trait. E.g., the stability parameters of R.I. Plaisted and L.C. Peterson [15] and G. Wrike [16) give preference to the 1/17-ob line (PP = 0.13; W^2 = 0.58), which forms a negligible number of fertile nodes with 2 pods per plant. However, the G. Annicchiarico' index [17] rated 22/16-ob (W_i = 99.45), Echo-af (W_i = 105.03) and B4-34 (W_i = 102.07) as the highest (Table 5). The PP and W^2 stability parameters confirm the approximate conclusion that can be drawn from the model of S.A. Eberhart and W.A. Russel [10]. In most cases, the coefficients bi of linear regression are positive, but in others they may have negative values due to causes of another nature (such as disease and pest infestation, a significant percentage of lodged plants). These reduce the coefficient values for the respective trait and lead to incorrectly formulated conclusions. The Shugar duarf variety is in such a situation for all four traits iassessed for the ecological stability.

The results obtained (see Table 4) for the reaction of samples of peas on the basis of the pods weight when changing the growing environment showed that a significant part of them had a very good responsiveness. In a favorable environment, these plants can be expected to form heavier pods. The varieties Plovdiv (b_i = 2.68), 1/17-ob (b_i = 2.63) and Marsy-n (b_i = 2.18) are the most plastic, which is characterized by the heaviest pods (56.48 g) (see Table 1) with statistically significant difference. The stability criterion S_i^2 indicates that Echo-af, Marsy-n and 22/16-af are relatively more stable than the other samples.

In the studied collection of pea specimens, according to the criteria presented in Table 5 afila type line 22/16-af and variety Echo-af have the smallest dispersion. The W_i [17] categorically ranks Marsy-n as the most unstable (W_i = 170.71), which from a breeding point of view is of interest due to the greater pods weight compared to other genotypes.

In terms of plant grain weight, it is noteworthy that high grain yields were characterized by the regression coefficient as the most variable with values of b_i = 2.62 (1/17-ob), b_i = 3.08 (Marsy-n), and b_i =4.02 (Plovdiv-n) (see Table 4). It can be assumed that Vechernitza-n and 22/16-af are close in stability and responsiveness to the ideal genotype with a b_i coefficient close to 1 and with a lower variance of the regression deviations. These specimens cannot take advantage of this because they occupy the lowest positions with respect to seed weight. Their parameters of stability and plasticity are nonsignificant and therefore their reaction to different environmental limits is unpredictable. The stability parameters PP and W^2 (see Table 5) showed that in the varieties Plovdiv-n and Shugar dwarf-n, the grain weight is very sensitive when the environmental conditions change. The assessment of these indicators as well as of W_i for the stability of the trait for 22/16-af and Vechernitza-n is unambiguous.

Table 6 report alternative approaches to assess the behavior of specimens grown in different environments. The rank analysis by the method of M. Huehn [20] makes it possible to assess the stability of certain genotypes in response to changes in the environment on the basis of their classification in different growing conditions. Line 22/16-ob ranked lowest (R = 2) in total number of pods, followed by 22/16-af, Echo-af, Marsy-n, and Vechernitza-n in second position (R = 4). According to the number of productive nodes with 2 pods per plant, only the 1/17-ob line has R = 3, and it is unpromising for this trait. Of the following samples in the ranking, the variety Marsy-n is of interest. Therefore, for breeding peas for an increased weight of the pod (with a sufficiently stable manifestation of the trait), we can recommend the variety Marsy-n and the line 22/16-ob, for the increased weight of grains per plant Marsy-n and the line 1/17-ob.

6. Phenotypic stability of the main productivity traits in the studied pea (*Pisum sativum* L.) samples based on non-parametric indicators (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020)

Sample	R, M. Huehn [20]				P_i, C.S. Lin и M.R. Binns [18]			
	NPP	NFN-2	WPP	WGP	NPP	NFN-2	WPP	WGP
22/16-ob	2	5	5	8	5.79	0.94	225.91	90.25
22/16-af	4	4	4	2	11.64	2.56	458.54	129.43
Kazino-af	7	7	7	6	10.18	2.13	348.16	93.81
Plovdiv-n	8	7	8	9	11.82	2.79	345.17	85.19
Echo-af	4	7	2	5	4.05	0.61	494.05	98.81
Marsy-n	4	4	4	6	1.56	0.18	391.31	103.86
Shugar dwarf-n	7	9	7	7	5.75	1.53	360.03	101.08
B4-34-n	6	5	8	7	5.90	0.83	396.39	142.23
1/17-ob	9	3	7	4	13.05	4.09	339.09	65.87
Vechernitza-n	4	4	4	2	16.08	2.71	554.49	128.14

Notиe. NPP means the number of pods per plant; NFN-2 means the number of fertile nodes with 2 pods per plant; WPP means total weight of pods per plant; WGP means grain weight per plant.

The P_i parameter by C.S. Lin and M.R. Binns [18] gives preference to the genotype with the lowest index. In terms of the total number of pods and number of fertile nodes with 2 pods per plant, the Echo-af and Marsy-n varieties, both also having high values of these traits, occupy the first two places by the stability. On the number of fertile nodes with 2 pods per plant, the B4-34 and 22/16-ob lines take the leading places. The index Pi for weight of pods and weight of grains gives priority to the lines 1/17-ob and 22/16-ob, as well as the variety Plovdiv-n.

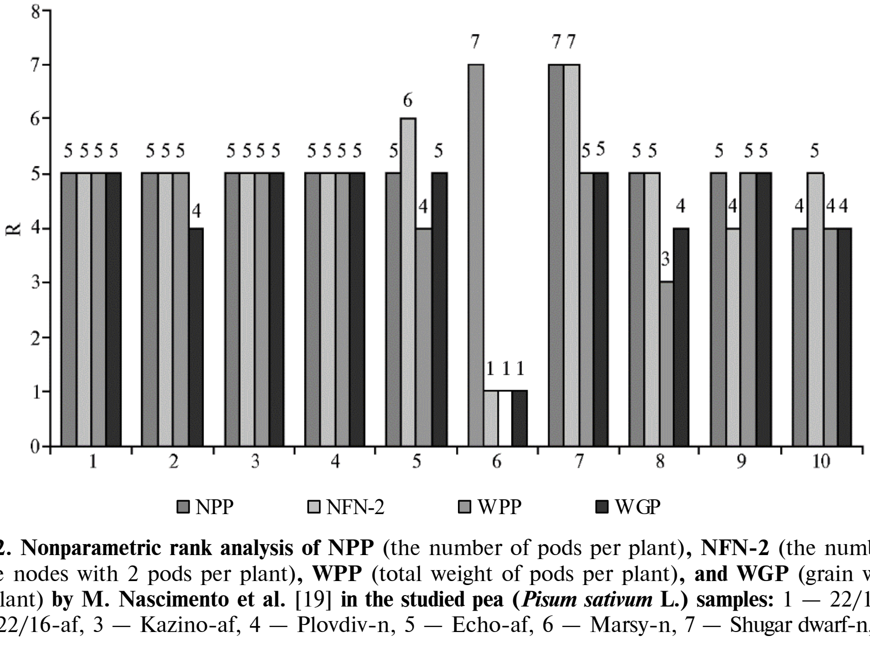

Fig. 2. Nonparametric rank analysis of NPP (the number of pods per plant)**, NFN-2** (the number of fertile nodes with 2 pods per plant)**, WPP** (total weight of pods per plant)**, and WGP** (grain weight per plant) **by M. Nascimento et al.** [19] **in the studied pea (*Pisum sativum* L.) samples:** 1 — 22/16-ob, 2 — 22/16-af, 3 — Kazino-af, 4 — Plovdiv-n, 5 — Echo-af, 6 — Marsy-n, 7 — Shugar dwarf-n, 8 —

B4-34-n, 9 — 1/17-ob, 10 — Vechernitza-n. For R ranks: 1 — high general adaptability; 2 — adaptability under favorable conditions; 3 — adaptability under unfavorable conditions; 4 — low adaptability; 5 — average general adaptability; 6 — adaptability under average favorable conditions; 7 — adaptability under average unfavorable conditions (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020).

According to the centroid classification method of M. Nascimento et al. [19] and the information obtained from Figure 2, a significant proportion of the pea samples showed average overall adaptability (R = 5) with respect to the total number of pods trait. Variety Vechernitza-n (R = 4) does not adapt well not only to this trait, but also to the weight of the pods and the weight of the grains of the plant. Marsy-n and Shugar dwarf-n perform relatively well in the adverse conditions. The Marsy-n variety is characterized by high overall adaptability (R = 1) on the other three grounds by the number of pods, number of fertile nodes with 2 pods per plant, the weight of the pods and the weight of the grains of a plant. Line B4-34 can be relied on to form sufficiently heavy pods (R = 3) when conditions are unfavorable, but not to feed heavy grains from a single plant. Some of the samples during the study period show average overall adaptability to the analyzed traits.

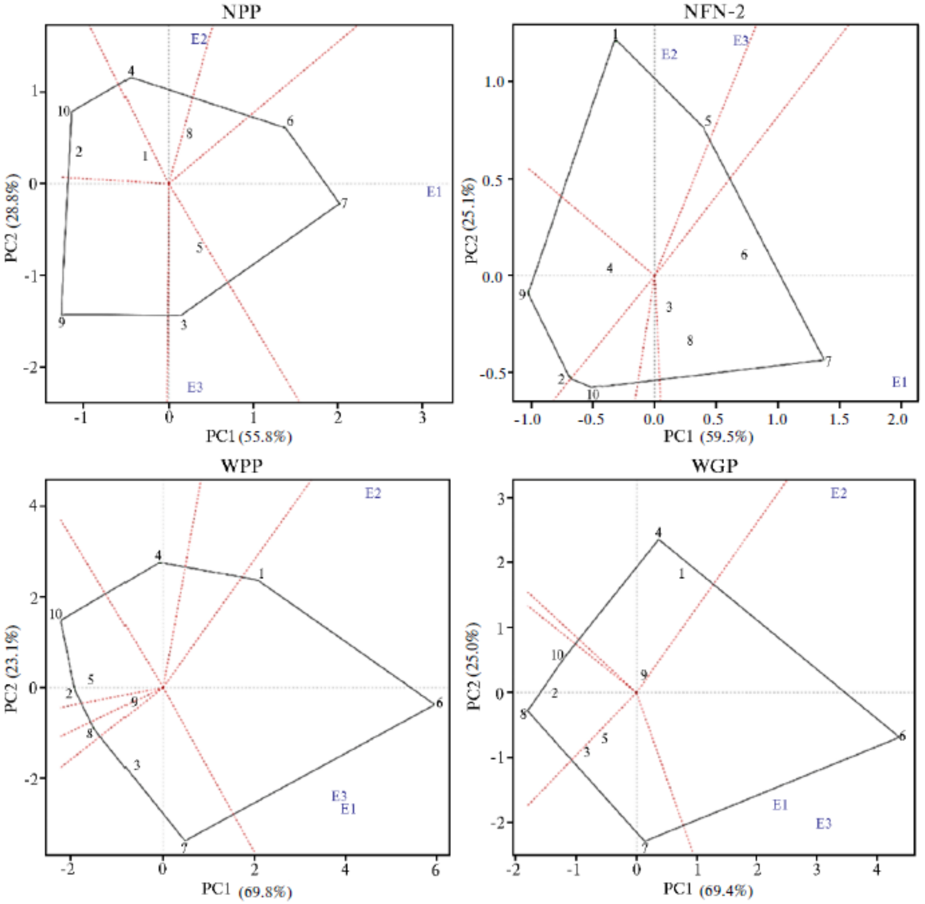

Рис. 3. Graphic of GGE biplot analysis of NPP (the number of pods per plant)**, NFN-2** (the number of fertile nodes with 2 pods per plant)**, WPP** (total weight of pods per plant)**, and WGP** (grain weight per plant) **traints in the studied pea (*Pisum sativum* L.) samples::** 1 — 22/16-ob, 2 — 22/16-af, 3 — Kazino-af, 4 — Plovdiv-n, 5 — Echo-af, 6 — Marsy-n, 7 — Shugar dwarf-n, 8 — B4-34-n, 9 — 1/17-ob, 10 — Vechernitza-n; E1, E2 and E3 — environmental conditions in 2018, 2019 and 2020, respectively (Maritsa Vegetable Crop Research Institute, Plovdiv, Bulgaria, 2018-2020).

GGE biplot analysis. GGE biplot is a complex analysis system designed to show most aspects of the genotype-environment interaction graphically.

The result of the experiment is presented in such a way that the visual evaluation of the samples and the identification of the "mega-environment" are significantly simplified. Only two principal components (PC1 and PC2) are preserved in the graphical model (Fig. 3), as this is the most appropriate way to establish the main patterns and to eliminate unnecessary data. The first two principal components can be plotted on 2D graphics so that the interaction between each genotype and the specific breeding environment can be easily interpreted on the biplot.

GGE biplot analysis for total pod number showed that the first twoprincipal components explained 84.6% of the total variability caused by the genotype-environment interaction. The Marsy-n and Shugar dwarf-n varieties can feed the largest number of pods in the E1 environment (2018), and the Plovdiv-n variety would give the best result in the E2 environment (2019). For Kazino-af and line 1/17-ob, the most suitable environment for the realization of a larger number of pods is E3 (2020), which is the most favorable for plant development compared to the rest of the study period (see Fig. 3).

On the basis of the number of fertile nodes with 2 pods per plant, a polygon is again formed, at the tops of which are the projections of the samples, which have an advantage in a certain environment (or group of environments). Line 22/16-ob and variety Echo-af thrive best in environments E2 (2019) and E3 (2020). Shugar dwarf-n, which as the previous genotype has a level of trait above the average for the studied sample, is positively affected by the climatic conditions of the environment E1 (2019). Genotypes Plovdiv-n, 1/17-ob, Vechernitza-n and 22/16-af are in the sectors without a specific environment and therefore in terms of adaptability of this trait they are inferior to the others (see Fig. 3).

The vertices of the polygon, graphically representing the behavior of the samples in the environment by the weight of the pods per plant, consist of the genotypes Plovdiv-n, occupying the top of the polygon, Vechernitza-n, 22/16-af. and 8-B4-34 located on the left, Shugar dwarf-n with a projection at the top, located at the bottom position and 22/16-ob and Marsy-n, which are on the right side of the polygon. The last two genotypes, especially Marsy-n, manage to form and feed more pods than the others. The Marsy-n variety is able to more fully realize this quality in the nearby first and third environments (E1, E3), while 22/16-ob develops better in E2 environments.

From the GGE biplot analysis of grain weight presented in Figure 3, some similarity was observed, both in the location of the samples and in the environment. It can be seen that in the polygon thus formed, the genotypes of peas are divided into five sectors. The Plovdiv-n, B4-34, Shugar dwarf-n and Marsy-n specimens are located at the tops of the landfill. The Marsy-n variety occupies an extreme right position, defined by a quadrant with a positive value of PC1 and a negative value of PC1, but not very far from the abscissa. This situation is due to the strong superiority of this variety over the others in the total weight of the grains. Among E1 and E3 are located very close to each other and form a mega environment. Environment E2 is in the same sector, but is located at its opposite end.

Threrof, in the study, the three sources of variation, i.e. genotype, environment and genotype-environment interaction, were found statistically significant for the total number of pods, number of fertile nodes with 2 pods per plant, pods weight and grain weight. Similar results in the same crop were previously reported [24, 25], especially with regard to the significance for the seed productivity indicator per plant.

Similar results were obtained in the study of chickpea genotypes [26]. The analysis of variance for pod length showed that more than 60% of the total trait variation was due to the influence of the growing environment, followed by the genotype-environment interaction. The genotype factor had the least influence on the manifestation of the trait. The findings of A.K. Mukherjee et al. [27] when testing rice varieties are in agreement with those from the study. The authors found that for a small part of the traits the greater sum of the squares for the genotype factor was obtained, from which it can be concluded that the studied samples of them differ significantly in their genetic talents. The influence of other factors of variation was weaker, especially for the genotype-environment interaction. Applying the method of S.A. Eberhart and W.A. Russel [10] to assess the phenotypic stability of quantitative traits in peas, C. Rana et al. [28] obtained similar results and reported statistical significance of the genotype-environment factor for the pods weight and plant seeds. When evaluating genotypes of Pannonian vetch [29] and garden peas [30] the authors report that samples with low seed productivity usually have high trait stability and specific adaptation to different growing conditions. The results in the present study support these findings. Based on the results of their practical experience with peas, Y. Goa and H. Mohammed [31] believe that the most appropriate and desirable genotype is one that combines high productivity and relatively good stability among the tested samples. According to the authors, the most highly productive genotypes are ecologically unstable with negative changes in environmental limits, but responsive under favorable environmental conditions [31]. Their statement is in line with the results of our study.

Our results on the stability of pea varieties with different leaf morphology are in agreement with previous similar studies, such as those of E. Acikgoz et al. [32]. The authors report that genotypes with afila leaf type type of leaves on the basis of plant seed weight are more stable than leaf forms, but the latter are more productive and are therefore preferred for cultivation in different environments.

In our study, a small number of pea samples combined high value and adequate stability of the respective trait. In other crops, such a pattern has also been established. In cowpea, T. Simion et al. [33] reported that a small number of genotypes analyzed by the main quantitative traits showed stability and high expression of the trait. The authors suggested that these genotypes would respond proportionally to changes in the rearing environment.

Y. Rezene et al. [34] in their work with peas reported that the GGE biplot analysis provided additional information about the studied varieties and their future practical use, which is confirmed in our study. In recent years, the GGE biplot technique has been widely used to study the genotype-environment interaction and stability in other crops such as soybeans [35], cowpea [36], chickpeas [37] and barley [38]. O. Sozena et al. [39] recommended that different methods and analyzes should be used to assess the phenotypic stability of the traits in order to obtain a more complete and accurate information about the studied plants. The results of their study show that parametric stability tests are appropriate and reliable.

Thus, we found the statistically significant influence of all factors of variation on such traits as total number of pods, number of fertile nodes with 2 pods per plant, pod weight and grain weight. The strongest effect of the environmental factor was observed for the total number of pods (52.20%) and the number of fertile nodes with 2 pods per plant (59.00%). The genotype factor has the largest part of the total variability of the weight of pods per plant (64.10%) and grain weight per plant (67.40%) traits. Therefore, an effective breeding can be done for improvement of these traits regardless of the environmental conditions. The number of pods per plant and pod length requires longer trials to obtain a more realistic estimate due to the superiority of the genotype×environment interaction variance over the genotype variance. Several stability parameters were calculated for each trait. The varieties Marsy-n and Echo-af have been identified as the most valuable genotypes for the number of pods per plant. Kazino-af, 1/17-ob and Plovdiv-n are highly variable and with a smaller number of pods. For pod weight, genotypes showed good responsiveness, especially Plovdiv-n ($b_i = 2.68$), 1/17-ob ($b_i = 2.63$) and Marsy-n ($b_i = 2.18$), which have a higher pod weight, while the Echo-af variety has better stability ($b_i = 1.39$; $S_i^2 = 1.91$). For the grain weight per plant, the highly productive samples were the Marsy-n ($b_i = 3.08$), 1/17-ob ($b_i = 2.62$), Plovdiv-n ($b_i = 4.02$), but these appeared most variable as well. Vechernitza-n and 22/16-af are close to the ideal genotype with regard to the stability, but are low-yielding.

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